the 72-hour period, without significant differences between 4°C and RT storage (29% and 20% initial values, respectively). T-cell content remained stable at both temperatures and in blood and BM HPC grafts during 3 days storage. A marked decline in the number of viable type 2 dendritic cell progenitors (DC2p) was seen in the first 24 hours in blood HPC grafts during storage at both 4°C and RT (27% and 24% and of initial values, respectively), while content of DC2p in BM HPC grafts remained stable up to 48 hours (60% and 70% initial values at 4°C and RT, respectively). Conclusions: Storage at 4°C did not impair the content of viable CD34+ cells or T-cells in blood or BM HPC grafts, and was superior to storage at RT in preserving the overall viability of blood HPC grafts. DC2p are particularly sensitive to storage, and prolonged transit times may impact the content of these immuno-regulatory cells in transplants.

Analyses after 72 hours: Median percentages of initial values	4 degrees BM HPC	Room Temp BM HPC	4 degrees Blood HPC	Room Temp Blood HPC
Viable CD34+	50%	29%	29%	20%
Viable CD3+	51%	63%	77%	27%
Viable DC2p	54%	31%	8%	11%

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OUTCOME OF PATIENTS TREATED WITH HIGH DOSE MELPHALAN WITH OR WITHOUT TBI FOLLOWED BY CD34 SELECTED STEM CELL RESCUE USING THE BAXTER ISOLEX 300I

Broun, E.R.; Sigmund, D.; Essell, J.; Tierney, C. Jewish Hospital, Cincinnati, OH.

We treated 24 patients with multiple myeloma with high dose melphalan +/- TBI using CD34 selection between 1/99 and 7/01. Patients were treated as part of initial therapy following 4 cycles of VAD in most cases. Mobilization was with either VP16/cyclophosphamide (5) or cyclophosphamide (19) followed by G-CSF. Patients then underwent 20L apheresis on a COBE Spectra with a collection goal of 6.5 x 10^6 CD34+ cells/kg. CD34 selection with the BAXTER Isolex 300i was carried out on the pheresis product with 1.5 x 10^6 CD34+cells/kg stored as backup. Patients underwent a median of 2 phereses (range 1-6) with a mean collection of 24.1 x 10 ^{6}CD34+ cells/kg (range 6.9-57.8). Following selection, a mean of 10.4 x 10^6 CD34+ cells/kg were infused at time of transplant. Post infusion recovery of WBC to an ANC >500/uL occurred in a mean of 26.9d (range 13-71d) and platelets to >20, 000/uL at 79.3d (range 15-360d). 4 patients expired prior to recovery of platelets and one remains platelet transfusion dependent 3 years post transplant. There was one early death due to aspiration pneumonia. Overall 10 pts have expired, cause of death: progressive disease 6, pneumonia 2, sepsis 2. The remaining 14 pts are alive, 3 in relapse and 11 (46%) in remission. Nine patients have relapsed (38%). Mean follow-up for the entire group is 18.4 mos. For the 11 pts in remission the median time from transplant is 18 mos (14-41 mos). CD34 selection led to prolonged recovery of granulocytes and platelets. Longer follow-up is needed to assess the durability of the remissions.

HEMATOPOIESIS/MESENCHYMAL CELLS

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ENGRAFTMENT AND PRODUCTION OF MESENCHYMAL COLONY FORMING CELLS (M-CFC) IN VITRO

La Russa, V.F.; Lee, M.; Price, S.; Safah, H.; Miller, A.; Rozans, M.; Weiner, R. TULANE UNIVERSITY CANCER CENTER, NEW ORLEANS, LA.

A 2-stage stromal cell culture system that supports production of early hematopoietic progenitor cells (LTCIC, CFU-BLAST) was evaluated for mesenchymal progenitor cells, M-CFC. Stage-1 was initiated by culturing female bone marrow low density mononuclear cells (LDMNC) for 2 weeks. At day 0, 2-week old female recipient stromal colonies were lethally irradiated (25Gy) to eliminate endogenous M-CFC(XX) and then seeded with 2.5X10^5 LDMNC from male-donor bone marrow containing only 27±3 M-CFC(XY). Engraftment and subsequent production of male donor-derived M-CFC (XY) activity was measured weekly by secondary plating of both non-adherent and adherent cell fractions derived from female-recipient stromal colonies that were initially seeded with male donor bone marrow LDMNC. Fluorescence in situ-hybridization (FISH) with DNA probes DXZI from the X-chromosome centromere region and the DYZI from the Yq12 region was performed on representative secondary M-CFC derived stromal colonies at each week . At week-1 there was >7X expansion of M-CFC(XY) activity in both adherent and non-adherent stromal cell fractions as compared to the original 27±3 M-CFC(XY) added at day 0. Most of the M-CFC(XY) activity localized within the female recipient adherent stromal cell fraction contained 187±17 M-CFC(XY)/plate and formed individual stromal colonies with surface area range: 4mm^2; <16 mm^2. In addition, there were 20±2 M-CFC(XY)/plate, that were more proliferative and formed larger colonies with surface area range: >16 mm^2; < 30 mm^2, and 10±2 M-CFC(XY)/plate with the highest proliferative potential forming colonies with surface area: >30 mm^2. At week-2, almost all of the M-CFC(XY) remained in direct-contact with the female recipient adherent stromal cell fraction and continued to further expand to 490±50 M-CFC(XY)/plate. The plating efficiency of mesenchymal progenitor cells at 3-weeks was >90% with a yield of 1, 150±150 M-CFC (XY) / plate, but their proliferative index was reduced to form smaller stromal cell colonies with surface area 4mm^2; <16 mm^2, and 13, 600±770 stromal cell clusters (XY)/plate. These studies show that lethally irradiated female recipient mesenchymal/stromal cell colonies support the engraftment of pre-clonogenic mesenchymal stem cells.

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FLOW CYTOMETRIC ANALYSIS OF CELLULAR DIFFERENTIATION ARREST IN MYELODYSPLASTIC SYNDROME

Sherman, M.; Dicke, K.; Palmer, D. Stem Cell Lab, Arlington Cancer Center, Arlington, TX.

CD33 has been shown to be a marker for cellular immaturity at the promyelocyte level. One of the hematological findings in myelodysplastic syndrome (MDS) is cellular differentiation (maturation) arrest. The purpose of this study was to determine the diagnostic utility of flow cytometry in confirming the clinical findings in MDS. Our study analyzed the percentage of CD33+ and CD33+/CD11c+ cells by flow cytometry in 18 patients clinically diagnosed with MDS. Controls consisted of 30 patients who were hematologically normal. Normal ranges for CD33+ and CD33+/CD11c+ were 19-41% and 58-80%, respectively. All 18 MDS patients were found to have a percentage of CD33+ cells in excess of 41% (43-68%) and a percentage of CD33+/CD11c+ cells less than 58% (31-56%), clearly demonstrating an accumulation of cells at the promyelocyte level at the expense of mature cells, demonstrating maturation arrest. We also found a slight increase in CD34+/CD33+ cells (from 0.5% to 0.73%), therefore a prelude for excess blast cells and the possible transformation of MDS into acute myelocytic leukemia (AML). We conclude that flow cytometry is a promising tool in assisting the clinician in diagnosing MDS and its progression.

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MOBILIZATION OF PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) WITH COMBINATION OF CYTOKINES IN AUTOLOGOUS AND ALLOGÉNEIC PEDIATRIC TRANSPLANT

Guerra, J.A.; Martinez, C.A.; Barrios, N.J. Pediatrics, University of Puerto Rico School of Medicine, San Juan, Puerto Rico.

Background: Clinical trials to mobilize PBPC for autologous and allogeneic harvesting prior to high dose chemotherapy / radiotherapy include chemotherapy, cytokines, or chemotherapy combined with cytokines. PBPC mobilized by G-CSF or GM-